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Modeling Insect Resistance to Insecticides Using Velvetbean Caterpillar (*Anticarsia gemmatalis*) as an Example‡

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Abstract: Evolution of insect resistance to insecticides, using the velvetbean caterpillar (*Anticarsia gemmatalis*) as an example, was evaluated with the help of a computer simulation model. The effects of the following factors were studied: mortality rate, dominance and initial frequency of the resistant gene, migration rate, reproductive disadvantage and action level. According to results of the model, mortality rate, genetic dominance and frequency, and insect migration were the most critical factors involved with the rate of evolution of insect resistance to insecticides. Mortality rate can be directly managed, and migration indirectly through preservation of refugia, as part of a program of resistance management, which should include insecticides with different modes of action on the insect, and at minimum efficient rates. © 1998 SCI

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Key words: mathematical model; genetic frequency; mortality rate; dominance; migration rate; resistance

1 INTRODUCTION

The development of insect resistance is an unwanted effect of insecticides. Since the first described case,[1] resistance had increased to 400 species in 1983,[2] and estimations were made that, by 1993, the number would be dangerously close to 1500, which is the number of major pests of world agriculture.[3] Even biological-based insecticides such as formulated *Baculovirus anticarsia* have proved to be able to induce strains of resistant hosts,[4] although surveys of field populations of the velvetbean caterpillar (*Anticarsia gemmatalis* Hübner) in different Brazilian and American locations did not show differences in LC_{50} values.[5] The practical importance of insect resistance is due to (a) reduction in the period for which a given insecticide can be used, increasing costs of R&D and also farmers' costs; (b) increase in insecticide field rates for successful control, with consequent increase in non-target effects; (c) insect control becoming very difficult, sometimes impossible; (d) increasing costs of production, of public and private scientific research and of environmental protection.

The major number of resistance cases described on the literature are in the United States and Japan, where investigations have established the biochemical basis of resistance, showing that alterations in the metabolic level of the insect, reduction of the insecticide penetration rate and biochemical modifications on the insecticide site of action are the most important factors. Metabolic activity studies indicated eight major pathways linked to higher levels of insecticide resistance: (a) action of mixed-function oxidase enzymes,[6,7] (b) P-450 cytochrome, a mono-oxygenase enzymatic system linked to degradation of exotic molecules;[8–10] induction of increased activity of P-450 has also been described,[11,12] (c) action of hydrolases and transferase enzymes, such as glutathione *S*-transferase,[13,14] (d)

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action of esterase enzymes on weak bonds of the insecticide molecule;[15] a relationship between resistance to organophosphorus and a highly active esterase has been established;[16,17] (e) enzymatic induction,[18] (f) reduced sensitivity of insect acetyl-cholinesterase;[19] (g) reduced sensitivity of nervous system, with changes in sodium channels and electrical properties of the membrane,[20] (h) reduction of insecticide penetration and/or binding due to physical-chemical barriers at the site of action.[21]

All these biochemical processes are genetically controlled. Several genes linked to insect resistance have been identified, but only up to six genes have so far been found to be involved with insecticide resistance in any given insect strain.[22] These genes may also mutate, with frequencies estimated between $1:10^4$ to $1:10^5$, in *Drosophila melanogaster* Meig. under radiation,[23] and studies have been made on the inheritability of types of resistance to organophosphorous insecticides.[24,25]

There are several biotic and abiotic factors linked to insecticide resistance evolution. The most important genetic factors are number, frequency and dominance of the genes. Biological factors may be physiological (like the reproductive parameters) or behavioral (like migration or refugia). Operational factors refer to the insecticide (chemical composition, persistence) or application (rate, mode or time). The diversity of factors and their relationships make it difficult to integrate experimentation, but simulation models allow individual contributions to be tested. Also, sensitivity of the model to variation and integration of levels of each factor with others can be tested. Mathematical models can be simple ones, irrealistic, complex with intrinsic factors or complex with intrinsic and extrinsic factors.[26] Simple models deal with low numbers of factors and barely investigate relationships between them. Demonstration of the dependence of the development of resistance on the dominance of the genes was made using this type of model.[27] Irrealistic models use regression analysis, with experimental data to forecast non-investigated cases. Complex models aim to demonstrate mathematical relationships between levels and factors.

The main purpose of developing the simulation model described here was to investigate the possibility of evolution of insecticide resistance in major pests of soybean and ways of avoiding its development[28,29] under the conditions of Brazilian soybean IPM, as used by the farmers.

2 METHODS

2.1 General aspects of the model

Evolution of insect resistance to insecticides as evaluated through the frequency of R gene, which confers the resistance trait to the insect, was studied using a mathematical model developed in BASIC (GWBASIC dialect), running on an IBM®-PC Computer, using double-precision variables when needed. Simplified unifactorial models were first designed to study the effects of individual factors, using lowest and highest possible levels of the tested factor, over the mean of the other factors. In this step the following factors were tested: (a) genetic (frequency, dominance and number of alleles, as well as previous selection by other insecticides with same mode of action); (b) biological (generation overlapping, reproductive capacity, migration, refugia and reproductive disadvantage of resistant specimens) and (c) operational (damage levels, mortality rate, timing of application and instar of the insect). For all biological, ecological, behavioral or operational parameters available results for *Anticarsia gemmatalis* Hübner (Lepidoptera: Noctuidae) the velvetbean caterpillar, were used.

Only the following factors, for which simple models showed sensitivity, were included in the definitive model: genetic frequency and dominance, mortality rate, migration rate, action level and reproductive disadvantage. Refugia proved to be critical for resistance evolution, but were not considered for simulation purposes, because the effects mimic the migration effect. Similarly, previous selection overlaps with gene frequency effects. The randomization method included the computer clock to avoid replication of population parameters in subsequent generations. The fluxogram of the definitive model is shown in Table 1.

Levels of factors were defined as lowest, highest and mean possible levels, except for the mortality rate, where practical standards were used. The lowest level of 50% represented an inefficient control of insects, and is useful to test the effect of low insecticide rates upon resistance evolution. The second level of 80% mortality represented Embrapa's official recommendation for soybean pest control, while 90% is the mean mortality rate observed on growers' field application. The highest rate of 99% represented the theoretical maximum, as the premise was accepted that the resistant R gene would not be eliminated from the insect population.

Initial genetic frequency levels of R gene were 0·01, 0·0001 and 0·000 001. No specific references to genetic frequency of R genes were found in the literature, and values were adapted from other traits. For genetic dominance, R fully dominant and fully recessive, as well as a mean value of 50% dominance were used. In spite of the present state of knowledge in this area, not demonstrating complete dominance of R genes, the sensitivity of the simple model to this factor made it interesting to explore the simulation of complete dominance.

Values of 40 (the official economic damage level),[30] 80 and four specimens per square meter were used to examine the effect of action level—i.e. the pest population which would trigger a treatment decision.

TABLE 1
Fluxogram of the Model

1. Start
   Memory partition
   Randomize
   Vectors and matrix dimensioning
2. Variables reading
   Mortality rate
   Dominance
   Frequency
   Migration
   Action level
   Reproductive disadvantage
3. Input parameters (keyboard)
4. Establish initial conditions
   Environmental capacity
   Reset generations
   Establish initial population
   Distribution by Genotypic class
   Print initial conditions
5. Control
   Establish mortality by genotypic class
   If susceptible mortality is greater than susceptibles
      Print MSG 'acquired resistance in Y generations'
      Go to 'increment levels'
   If not
      If population is too low to proceed
         Go to 9. 'increment level'
      If not
         Calculate mortality rates by class
6. Population balance
   If migration exists
      Calculate theoric migration (I)
      Calculate environmental space (II)
      Adjust (I) to (II)
      Recalculate individuals by class
   In both cases
      Recalculate specimens by class
      Recalculate genetic frequency
      If genetic frequency of R > 0·5
         Print MSG 'acquired resistance in Y generations'
         Print last genetic frequency
         Go to 'increment levels'
7. Reproduction
   Establish insect growth rate
   Increment insect population
   Adjust population to environment capacity
   If population is too low
      Print MSG 'population too low to proceed'
      Go to 'increment levels'
   Recalculate population by class
   Recalculate genetic frequency
   Generation count advance
   If reproductive disadvantage exists
      Establish correction factor for RR and RS
         Adjust SS class to environmental capacity
   If population has not reached action level
      Go to reproduction

TABLE 1 *Continued*

8. Balance
   Format variables for printing
   Print latest conditions
   Reset auxiliar variables
   If generation > 50
      Print MSG 'Resistance not acquired'
      Go to increment levels
   Prepare next loop
9. Increment levels
   Increment level of mortality
   If mortality level > larger level
      Increment frequency level
      Reset mortality rate
   If frequency level > larger level
      Increment dominance level
      Reset previous factors levels
   If dominance level > larger level
      Increment migration level
      Reset previous factors levels
   If migration level > than larger level
      Increment action level
      Reset previous factors levels
   If action level > large level
      Increment reproductive disadvantage
      Reset previous factors levels
   If reproductive disadvantage level > larger level
      End
   Go to 5. 'Control'

Linkage with practical aspects is based on the fact that some growers apply early in the season, with low insect populations, while others will apply later than recommended. An insecticide-resistant strain of *Culex quinquefasciatus* (Say) showed fecundity 35% lower than the susceptible strain,[31] representing a handicap for the resistance trait. For the present model the levels of 0, 25 and 50% of reproductive disadvantage were used. The migration process was presented by a stock population not affected by selection pressure, in-migrating to the model area just before reproduction. Levels of in-migration of 0, 50 and 100% of the model population, mating with the previous existing population, were considered.

2.2 Mathematical formulae

2.2.1 Insect mortality

Resistant homozygous (RR) mortality rate (MRR) was arbitrarily defined as being 20% of the rate for general population, meaning a resistant factor (r) of 5, which is a fairly good approximation of $r = 3$–30 found on other insects studied. Susceptible homozygous (SS) rate of mortality (MSS) was found by the difference between the general population mortality and the mortality of RR and RS groups. It was defined that SS are easier to

kill than the other groups, and if a certain degree of mortality was desired, the model would have to kill as many SS individuals as necessary to reach the mortality rate of the general population. The rationale of the system is the attribution of a rate-dependent mortality (MRS) for RS individuals. Heterozygotes respond to the insecticide rate depending on the dominance of the R gene.

$$\mathrm{MRS} = X(\mathrm{I}) \cdot (A_1 + (A_2 \cdot E(\mathrm{J}))) \quad (1)$$

where:

MRS = Rate of mortality for heterozygous (RS) (%)
X(I) = Rate of mortality for general populations (%)
A_1 = −0·125 (Intercept)
A_2 = −0·007 75 (Linear coefficient)
E(J) = Dominance level (0 if R dominant and 1 if R recessive)

The primary mortality equation as derived from experimental data from several insecticide tests for the control of *A. gemmatalis*.[32] Fitness calculations were made using mathematical equations seeking a non-linear relationship between dominance and mortality that would result in MRS slightly closer to MSS, in the absence of dominance of either R or S. The best fitness was achieved using quadratic regression models, in the desired (50–99%) interval, but more realistic results were obtained when very low values of linear and quadratic coefficients were tested. In this situation, results almost completely overlapped those obtained with a linear equation, and this last approach was adopted. The logic of this equation is that if R is dominant (E(J) = 0), the heterozygous mortality rate equals the resistant mortality rate. If the S gene is dominant, the model considers both groups (RS and SS) as having the same mortality rate. In the case of no dominance, RS mortality rate will be calculated by eqn (1) above.

2.2.2 *Genetic frequency*

The Hardy–Weinberg formula was used to establish initial genetic frequency for each genotypic class, as follows:

$$F_{\mathrm{RR}} = F\mathrm{A}^2 \quad (2)$$

$$F_{\mathrm{RS}} = 2 \cdot \mathrm{FA} \cdot (1 - F\mathrm{A}) \quad (3)$$

$$F_{\mathrm{SS}} = (1 - F\mathrm{A})^2 \quad (4)$$

where

F_{RR} = RR frequency
FA = R gene frequency
R_{RS} = RS frequency
F_{SS} = SS frequency

Once the initial genetic frequency was established, subsequent generation frequencies were iteratively calculated after applying mortality rates, through the following equation systems:

$$F\mathrm{A}_{n+1} = \frac{(S_{\mathrm{RR}} \cdot F\mathrm{A}_n^2) + (S_{\mathrm{RS}} \cdot F\mathrm{A}_n \cdot F\mathrm{S}_n)}{(S_{\mathrm{RR}} \cdot F\mathrm{A}_n^2) + (2 \cdot S_{\mathrm{RS}} \cdot F\mathrm{A}_n \cdot F\mathrm{S}_n) + (S_{\mathrm{SS}} \cdot F\mathrm{S}_n^2)} \quad (5)$$

$$F\mathrm{S}_{n+1} = \frac{(S_{\mathrm{RS}} \cdot F\mathrm{A}_n \cdot F\mathrm{S}_n) + (S_{\mathrm{SS}} \cdot F\mathrm{S}_n^2)}{(S_{\mathrm{RR}} \cdot F\mathrm{A}_n^2) + (2 \cdot S_{\mathrm{RS}} \cdot F\mathrm{A}_n \cdot F\mathrm{S}_n) + (S_{\mathrm{SS}} \cdot F\mathrm{S}_n^2)} \quad (6)$$

where:

$F\mathrm{A}_{n+1}$ = R frequency in next generation
$F\mathrm{S}_{n+1}$ = S frequency in next generation
$F\mathrm{A}_n$ = R frequency in present generation
$F\mathrm{S}_n$ = S frequency in present generation
S_{RR} = RR surviving
S_{RS} = RS surviving
S_{SS} = SS surviving

2.2.3 *Migration*

Migration rate was calculated as the percentage of the population that enter the model area immediately after insecticide application and mixed with the remaining population. Genetic frequency of the different classes followed the Hardy–Weinberg distribution, using the model initial frequency of R gene to locate total migrating population into genotypic classes:

$$PM = (PM_{\mathrm{RR}} \cdot F\mathrm{A}^2) + (2 \cdot PM_{\mathrm{RS}} \cdot F\mathrm{A} \cdot F\mathrm{S}) + (PM_{\mathrm{SS}} \cdot F\mathrm{S}^2) \quad (7)$$

where

FA = R gene frequency
FS = S gene frequency
PM = Total migrating population
PM_{RR} = RR migrating
PM_{RS} = RS migrating
PM_{SS} = SS migrating

Genotypic classes of migrating insects were added to the same classes of insects surviving the insecticide application, and the new genetic frequency was recalculated, through the following equation system:

$$F\mathrm{A}_n = \frac{(A \cdot F\mathrm{A}^2) + (B \cdot F\mathrm{A} \cdot F\mathrm{S})}{(A \cdot F\mathrm{A}^2) + (2 \cdot B \cdot F\mathrm{A} \cdot F\mathrm{S}) + (C \cdot F\mathrm{S}^2)} \quad (8)$$

$$FS_n = \frac{(C \cdot F\mathrm{S}^2) + (B \cdot F\mathrm{A} \cdot F\mathrm{S})}{(A \cdot F\mathrm{A}^2) + (2 \cdot B \cdot F\mathrm{A} \cdot F\mathrm{S}) + (C \cdot F\mathrm{S}^2)} \quad (9)$$

where

A = remaining plus migrating RR
B = remaining plus migrating RS
C = remaining plus migrating SS

Assuming no selection pressure on the migrating population, the major practical effect of migration was to dilute the frequency of the R gene in the combined population.

2.2.4 *Population balance*

The carrying capacity of the environment (K) was defined as $1{\cdot}6 \times 10^6$ ha^{-1}, equivalent to twice the highest pest pressure established, and of perfectly possible occurrence in field situations. For population balance, the model initially calculates the theoretical migration (PMT), applying migration rate on the present population, conditioned by environmental vacuum:

$$K_1 = K(RR + RS + SS) \tag{10}$$

where

K_1 = Environmental vacuum
K = Maximum carrying capacity of the environment
RR = Number of RR individuals
RS = Number of RS individuals
SS = Number of SS individuals

If PMT $> K_1$, the model forces PMT $= K_1$. The value of PMT is distributed into phenotypic classes and added to remaining population. After recalculating the genetic frequency, the specific R gene frequency is tested, and if $FA > 0{\cdot}5$, then the model reports the present generation and FA and prints a message of acquired resistance.

After incorporating migrating individuals, reproduction is applied, allowing the insects to mate freely. In simple versions of the model, the classic descending density-dependant approach was adopted, which says that the intrinsic rate of reproduction of a given species is inversely proportional to the population present at the moment reproduction is in process, limited to the maximum reproductive index of the species and the carrying capacity of the environment. The approach of the general model is:

$$PR = PA^{\mathrm{X(K-PA)/K}} \tag{11}$$

where

PR = Resulting population
PA = Prior population
X = Reproductive coefficient of the species
K = Environment carrying capacity

The general model did not fit well to *A. gemmatalis* biology and behavior, so studies were made to alter X, the only variable of the model (e.g. $X = (PA/N)^{-Y}$, $X = LOG_{PA}\ N$, $X = PA^{(1-N)/N}$, etc.), and to adjust the insect cycle to other mathematical fitness models (sine, monomolecular, logarithmic exponential, parabolic, Gompertz, hyperbolic), but none of these reflected field data with any accuracy. From the experimental data the following growth equation system could be deduced:

$$X_1 = ((X^2) \cdot B_3) + (X \cdot B_2) + B_1 \tag{12}$$

$$X_2 = 2{\cdot}718\,281\,828^{X_1} \tag{13}$$

$$\mathrm{AUX} = 1 - (\mathrm{RND} \cdot 0{\cdot}3) \tag{14}$$

$$PR = PA \cdot X_2 \cdot \mathrm{AUX} \tag{15}$$

where

X = Ln of the species growing rate ($X = \log(Y)$, $Y = 1$–5)
B_1, B_2 and B_3 = Polynomial coefficients
AUX = Randomization factor ($0 < \mathrm{AUX} < 0{\cdot}71$).

The randomization variable AUX stands for all biotic (natural enemies, food, etc.) or abiotic (weather, cultural practices) factors, which affect the insect population in the field, but do not interfere with resistance evolution, and its range was obtained after a series of iterative calculations. To maintain the classic density-dependent population growth, according to available field data for the insect, values of X (growing coefficient) were calculated at different insect population intervals. Table 2 shows population intervals and coefficients used. As the sensitivity of the model to the coefficients was high up to 10 insects m^{-2}, medium between 11–50 and low with more than 50 insects m^{-2}, intervals were not equally

TABLE 2
Growing Coefficients (X) by Population Intervals Measured in Insects per Square Meter

Interval	*Coefficient*	*Interval*	*Coefficient*
< 1·0	2·00	20·1–25·0	1·20
1·1–2·0	1·70	25·1–30·0	1·17
2·1–3·0	1·60	30·1–35·0	1·15
3·1–4·0	1·50	35·1–40·0	1·13
4·1–5·0	1·45	40·1–50·0	1.10
5·1–6·0	1·40	50·1–60·0	1·07
6·1–7·0	1·37	60·1–70·0	1·05
7·1–8·0	1·35	70·1–80·0	1·04
8·1–9·0	1·33	80·1–90·0	1·03
9·1–10·0	1·30	90·1–100·0	1·02
10·1–15·0	1·25	100·1–110·0	1·01
15·1–20·0	1·22	> 110·1	1·00

spaced. By fixing X = 1, the population tends to stabilize and not to grow by reproduction. That is the reason for the limit of 110 insects m^{-2}, lower than the carrying capacity of the environment (K = 160), which was intentionally established to allow a margin for migrating individuals to enter the model. By using data from Table 2, the growth equation system can be reduced to a simpler formula

$$PR/1000 = ((PA/1000)^{X_2}) \cdot \text{AUX} \qquad (15)$$

Division of PR and PA by 1000 serves solely to permit $1 \leq X_2 \leq 2$, avoiding the use of fractional coefficients. After calculation, the model reconverts insect population to the base area of one hectare.

The exit condition of the model was reaching the last level of the last factor (level 3 of reproductive disadvantage). To increase levels of the several factors involved, conditions were: (a) the model completed generation 50 without resistance evolution; (b) the insect became resistant; (c) the insect population was too low to enter the reproductive stage.

3 RESULTS AND DISCUSSION

3.1 Exploratory models

Thirteen factors possibly linked to insecticide resistance were studied using preliminary and individual models, aiming to test the sensitivity of the model to variation of level of each factor independently. The exception was the number of alleles and their dominance, which were tested together. To test each factor, its extreme but possible levels were tested, the other factors being constant at the mean level. Results of these simulations showed that eight out of 13 factors studied were critical for resistance evolution, the model results changing as the factor varied (Table 3). Prior selection proved to be mathematically equivalent to the initial genetic frequency in its formulation. In practical terms, prior selection or cross-resistance can be represented by levels of initial frequency. In the same sense, refugia can interfere with model outputs, but the mathematical formulation of this factor is very similar to that of migration and is represented by a population with different genetic frequency of R gene during the reproduction process. Polygeny can also be critical for the model. A combined preliminary exercise involving number of genes and dominance showed that dominance tended to eclipse polygenic effects. Hence, in order to reduce the algebraic complexity of the model, and especially to avoid the large number of possible combinations that would result, and which would be very difficult to analyze, the factors, previous selection, refugia and polygeny were not used in the definitive simulation model.

TABLE 3
Sensitivity Analysis of Preliminary Models to Several Factors Possibly Affecting Insect Resistance to Insecticides

Factors	*Level*[a] *N*[b] *1*	*2*	*Sensitivity*[c]
Number of genes	38	41	4
Frequency	30	48	1
Dominance	28	50	1
Generation overlapping	49	49	5
Previous selection	28	47	1
Gene mutation	48	48	5
Reproductive capacity	48	49	5
Migration	30	46	1
Refugia	29	44	1
Reproductive disadvantage	36	41	1
Damage level	37	40	3
Mortality rate	20	50	1
Insect age	48	48	5

[a] Level 1 = low; level 2 = high.
[b] Number of generations for insecticide resistance.
[c] Sensitivity 1 = high; 5 = low.

3.2 Definitive model

With number of possible generations limited to 50, in 82% of cases there was no evolution of insecticide resistance. The overall mean indicated that, in the velvetbean caterpillar, resistance to insecticides can be achieved in 39 generations. In 96% of the generations, an insecticide application was necessary, indicating that insects were exposed to 37 selection cycles. Considering the average number of insecticide applications under Brazilian conditions,[33] 19 years of consecutive use of the same insecticide, or insecticides acting on the same site in insect biochemistry, would be necessary for a given population to evolve resistance to the chemical.

The number of generations necessary to evolve resistance varied from a minimum of three to a maximum of 50, which was the limit allowed by the model (Table 4). When the resistance was achieved after three generations, the insect mortality rate was 99%, while a mortality rate of 50% was associated with evolving resistance in the 50th generation. For the other factors studied, the level was the same for resistance acquired after three or 50 generations, which shows that the field doses of insecticide (rate of mortality) provide the key to the likely development of resistance. High initial frequency (possibly due to past selection or cross-resistance), somewhat linked to field doses of the insecticide, is also very important, but the effect was the same in both cases.

The mean effects of the levels of each factor are presented in Fig. 1, clearly showing that mortality rate, initial R gene frequency, dominance and migration are

TABLE 4
Levels of Factors Associated with Minimum and Maximum Number of Generations Necessary for Development of Insecticide Resistance

Factors	*Levels*	
	3 gen	*50 gen*
Damage level (m^{-2})	4	4
Migration	0	0
Reproductive disadvantage (%)	50	50
Initial frequency of R	0·01	0·01
Dominance of R (%)	100	100
Mortality rate (%)	99	50

the most critical factors. For migration, the main contribution is due to its presence or absence more than to its level, while, with other factors, an almost linear relationship between levels and generations can be observed. The model was less sensitive to action level and reproductive disadvantage. Mathematical relationships between levels within each factor and number of generations for resistance are presented in Table 5.

The adjusted relationship between mortality rate and number of generations is shown in Fig. 2. The linear component of the equation ($Y = 66{\cdot}45 - 0{\cdot}32X$; $r^2 = 0{\cdot}96$) allows one to state that, in statistical terms, for each 3% increase in mortality rate, the number of generations necessary to develop resistance is reduced by one. Calculating the maximum level of this factor over the mean level of others, the model indicated that 28 generations were necessary to achieve resistance. The general results agree with other similar studies showing the inverse relationship between mortality rate and number of generations to achieve resistance.[34,35]

Figure 3 shows the relationship between R frequency and the number of generations for resistance evolution, while an example of evolution of R and S frequency and insect population is shown in Fig. 4. If R is fully recessive, it is necessary for other conditions to be very favorable for the insects to develop resistance, as the model demonstrated high sensitivity to this factor (Fig. 5), as has also been established by other authors.[36] In spite of the best fit for this factor being a second-order equation, the linear component had the strongest participation (Table 5). Generally, for each six points of increment of the level of dominance, one less generation is required

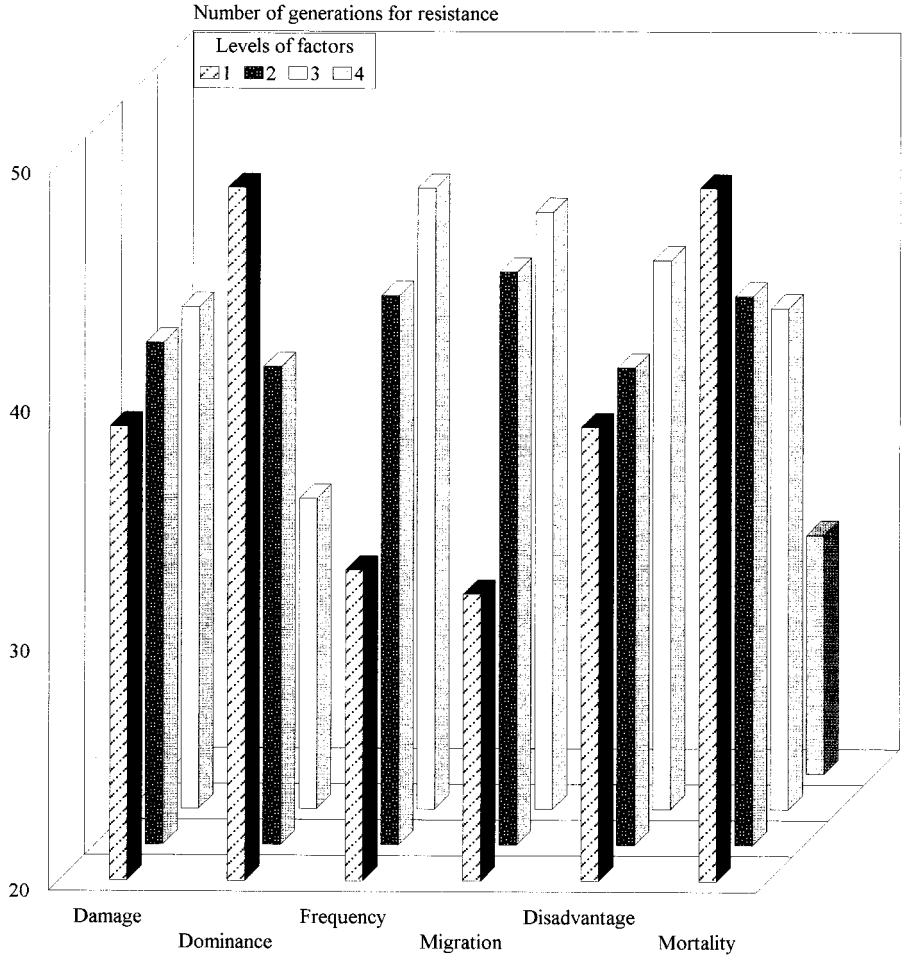

Fig. 1. Factors involved with insect resistance to insecticides used in the definitive model, and its relationship to resistance development. The level of each factor was calculated over the mean of the others.

TABLE 5
Equations Relating Factors and Number of Generations Necessary for Development of Insecticide Resistance[a]

Factor	*Equation*
Damage level	$Y = 39{\cdot}09 + 0{\cdot}053X + 0{\cdot}0031X^2$
Reproductive disadvantage	$Y = 42{\cdot}7 + 13{\cdot}6X - 11{\cdot}2X^2$
Genetic frequency	$Y = 46{\cdot}37e(901{\cdot}38X + 390\,122X^2 - 3 \times 10^{-7}X^3)$
Dominance	$Y = 48{\cdot}9 - 0{\cdot}18X + 0{\cdot}0002X^2$
Migration	$Y = 32 + 0{\cdot}73X - 0{\cdot}0092X^2$
Mortality rate	$Y = 2\,772\,914e(-0{\cdot}48X + 0{\cdot}006X^2 - 3 \times 10^{-5}X^3)$

[a] The best fit was considered the lower grade equation with significant parameters and higher r^2.

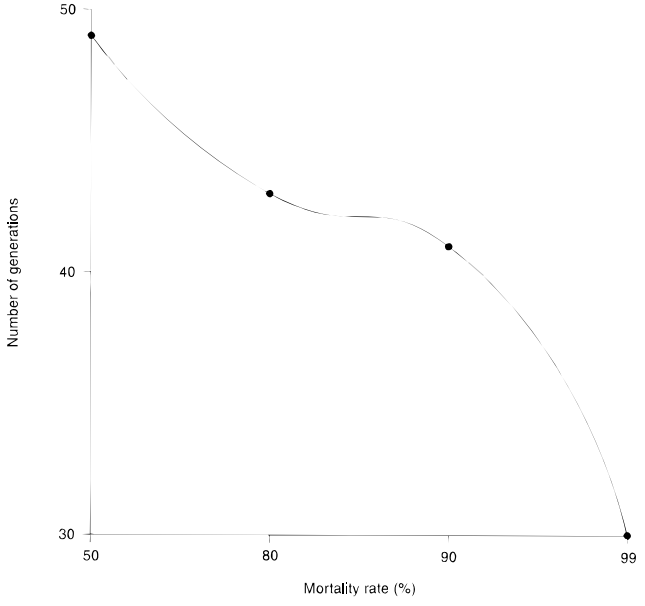

Fig. 2. Relationship between mortality rate and number of generations for evolution of insecticide resistance.

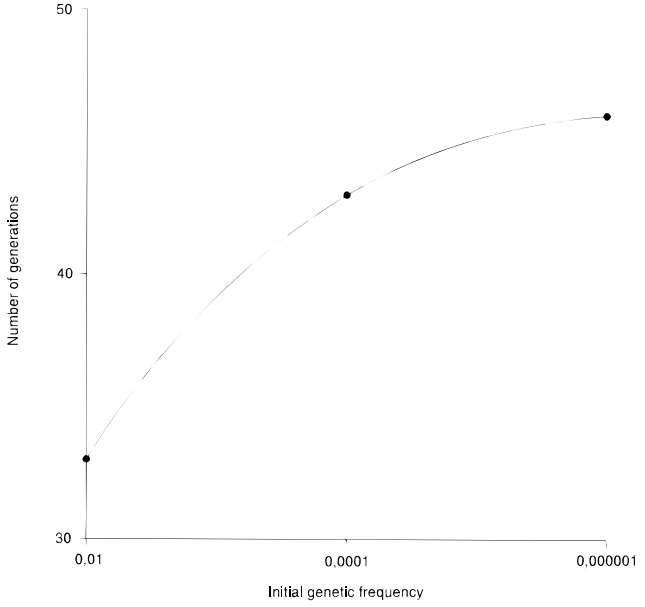

Fig. 3. Relationship between initial genetic frequency and number of generations for evolution of insecticide resistance.

for the appearance of insecticide resistance, the model being more sensitive to this factor than to the genetic frequency, making dominance the second most important factor for developing insecticide resistance.

Migration rate relates exponentially with number of generations (Fig. 6). The negative sign of the quadratic component of the equation (Table 5) indicated that migration interference in the model tended to stabilize after a point between the levels of 25 and 50%. Reorganization of the genetic pool proved to be deleterious for resistance evolution, confirming studies made with other insects.[36–38]

One hypothesis used to formulate the simulation model was that reduction of action level would imply greater use of insecticide, putting more selection pressure upon the insect population, hence accelerating resistance to insecticides. Use of 40 or 80 insects m^{-2} showed only a moderate contribution to the model. Nevertheless, due to its practical importance, and as it is one of the factors in resistance management, a very low level of 4 m^{-2} was included in the definitive model, to test its interaction with levels of other factors. Results showed that, in spite of a relation of 1 : 20 between lower and higher levels, the number of generations necessary for insecticide resistance was reduced by less than 5%, calculated upon the mean of other factors. The main contribution was observed between levels 1 and 2, and there was no practical difference between levels 2 and 3 (Fig. 7).

Reproductive disadvantage showed only a small impact on the outputs of the model, as the difference between extreme levels resulted in a reduction of only three generations for resistance (Fig. 8). The mathematical relationship (Table 5) showed that the highest levels of disadvantage may lead to different results, as found in other studies, with high impact at 60% level, avoiding elimination of the S gene from the population.[38]

Six sample cases of the model output, out of 943 studied, are presented in Figs 9 to 14. The first case shows the importance of the combinations containing almost all the most suitable conditions for developing

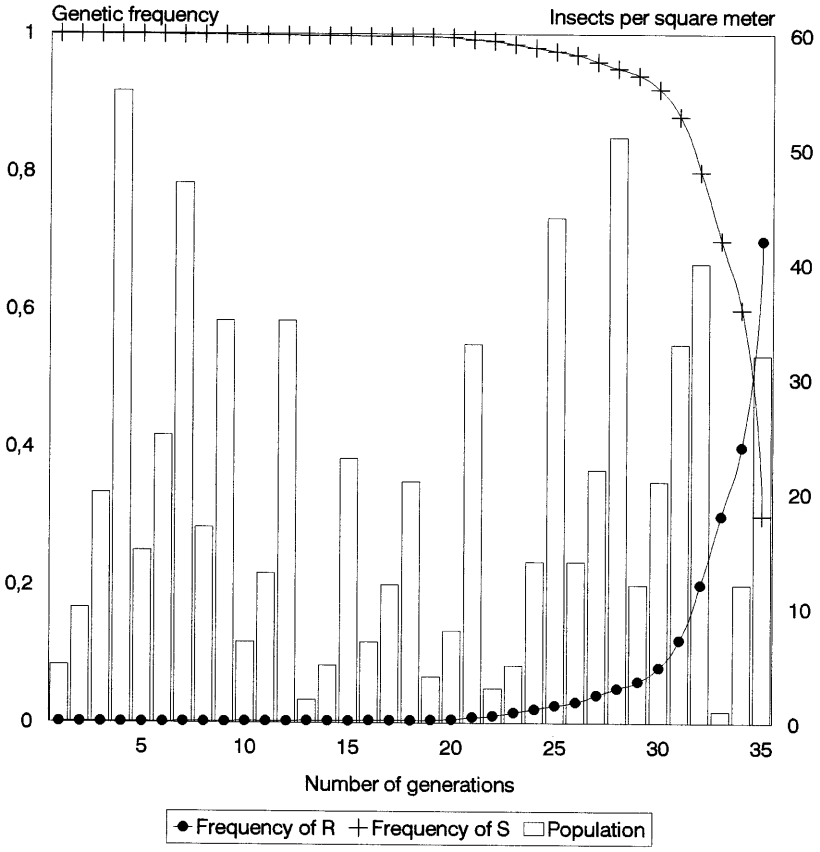

Fig. 4. Evolution of insect population and genetic frequency of R and S genes—case study.

insect resistance, which are no insect migration, higher reproductive disadvantage, and a lower action level, leading to more frequent insecticide application. In this case resistance can be achieved in only two generations, when high mortality rate is applied in combination with high genetic frequency and with R fully dominant (Fig. 9). Combination of higher (F_1) and intermediate (F_2) genetic frequency, with full dominance of R gene always resulted in insect resistance, even at low selection pressure.

Introducing 50% insect migration and considering no reproductive disadvantage, only the highest genetic frequency, with intermediate or high level of R dominance would result in development of resistance (Fig. 10).

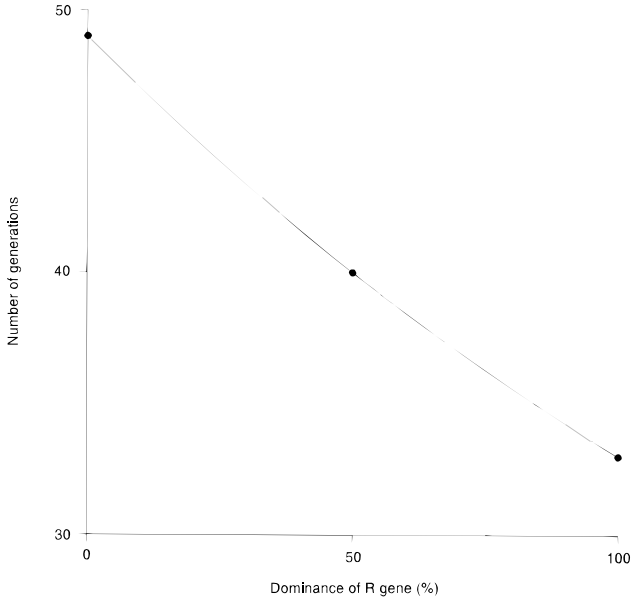

Fig. 5. Relationship between genetic dominance and number of generations for evolution of insecticide resistance.

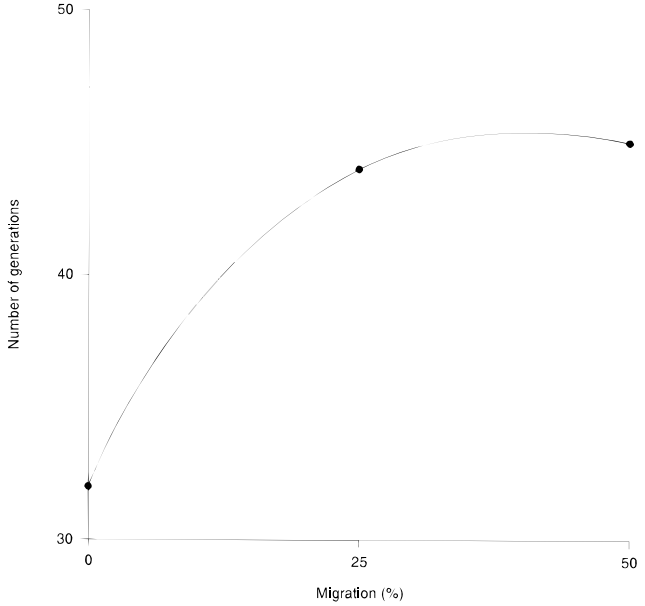

Fig. 6. Relationship between migration rate and number of generations for evolution of insecticide resistance.

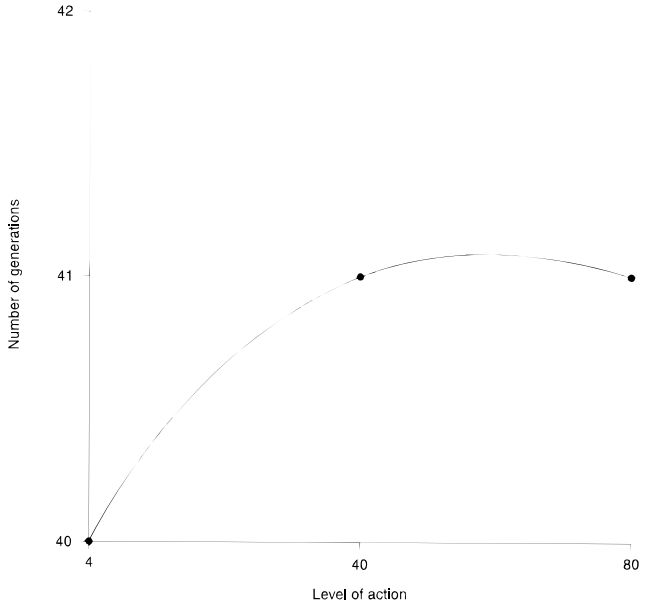

Fig. 7. Relationship between level of action and number of generations for evolution of insecticide resistance.

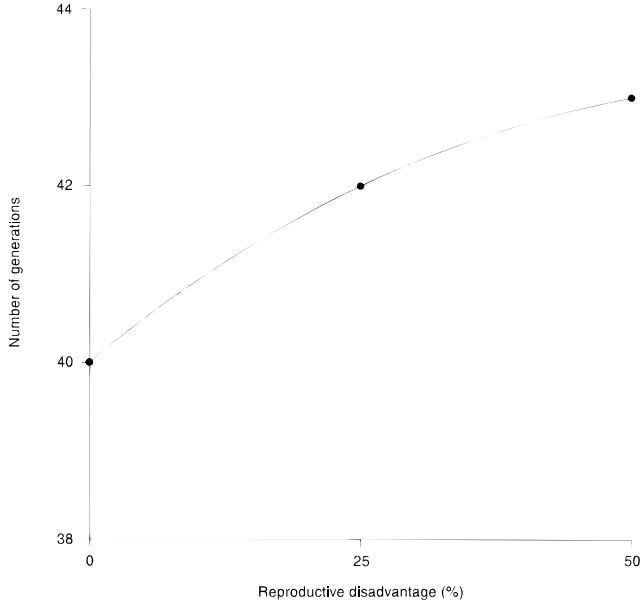

Fig. 8. Relationship between reproductive disadvantage and number of generations for evolution of insecticide resistance.

Changing only the migration level, from 50 to 100% of the insect population, does not alter the model outputs (Fig. 11), demonstrating that gene exchange is important, but it is not necessary to have the same density of unchallenged and selected insect populations to avoid insecticide resistance, as is clearly shown also in Fig. 6.

The effect of changing the reproductive disadvantage from higher level (3) to intermediate level (2) is evident from the results of the case study shown in Fig. 12, as compared to Fig. 11. In this case, resistance can be reached even with intermediate genetic frequency (0.0001), when R is fully dominant.

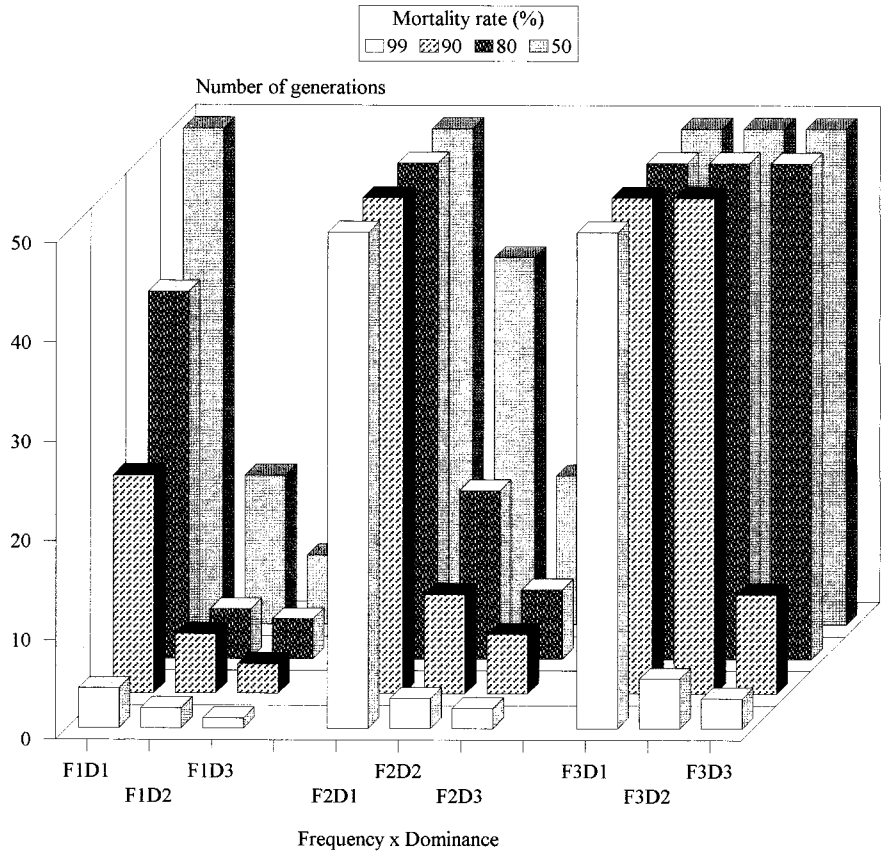

Fig. 9. Number of generations for evolution of insecticide resistance considering level 1 of action level, migration and reproductive disadvantage. Case study 1.

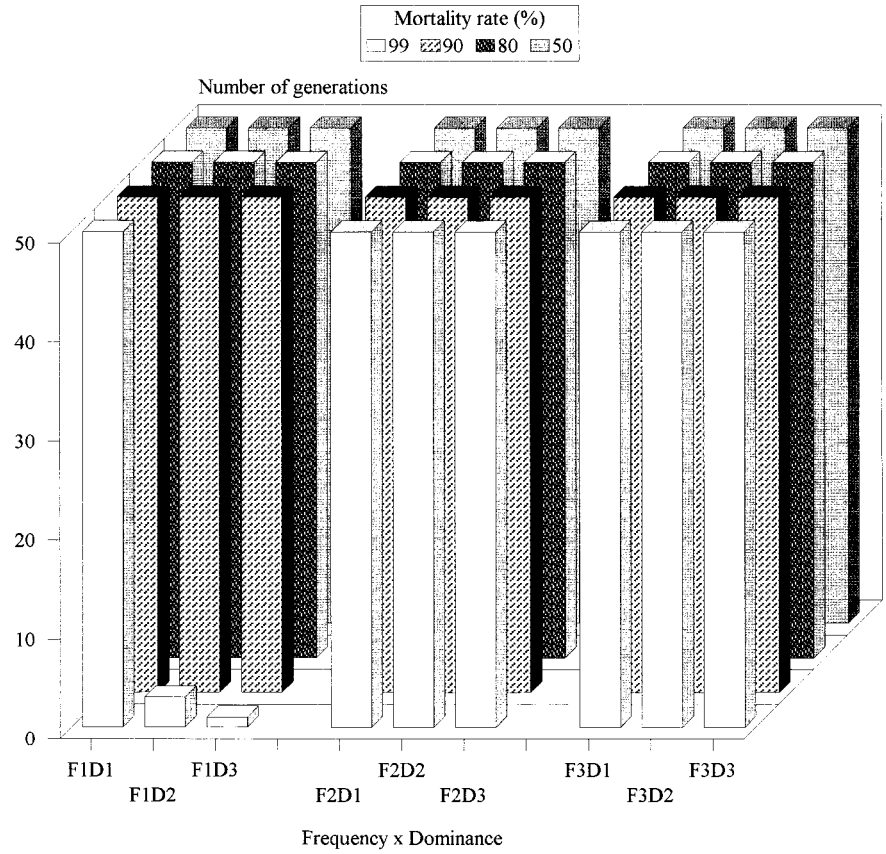

Fig. 10. Number of generations for evolution of insecticide resistance considering level 1 of action level, level 2 of migration and level 3 of reproductive disadvantage. Case study 2.

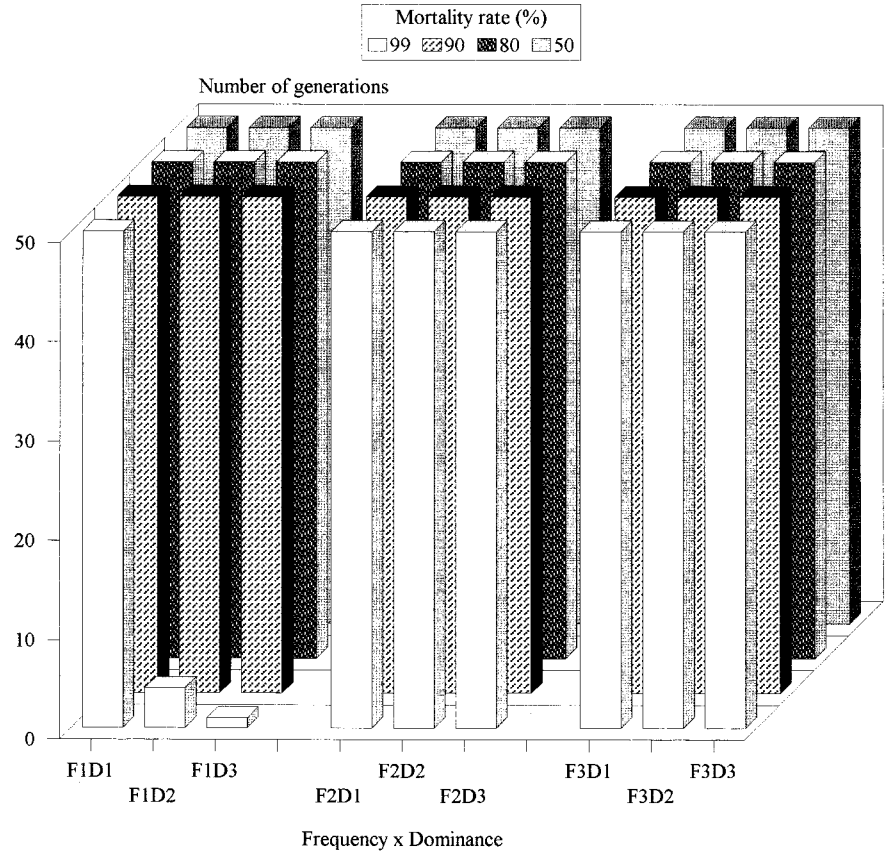

Fig. 11. Number of generations for evolution of insecticide resistance considering level 1 of action level, level 3 of migration and level 3 of reproductive disadvantage. Case study 3.

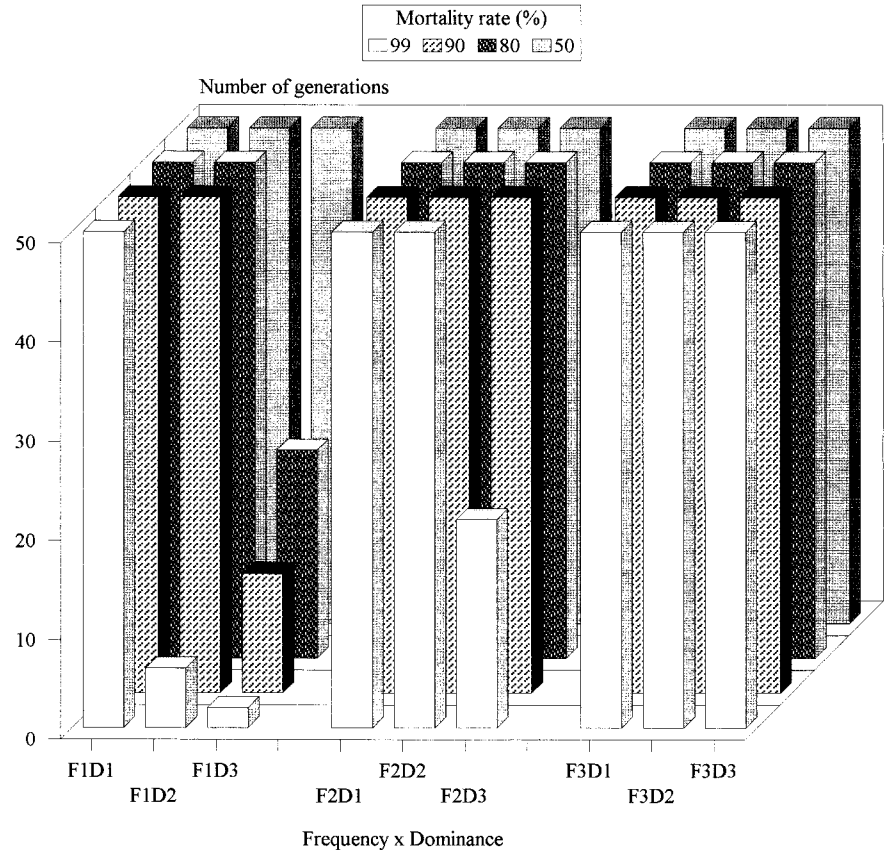

Fig. 12. Number of generations for evolution of insecticide resistance considering level 2 of action level, migration and reproductive disadvantage. Case study 4.

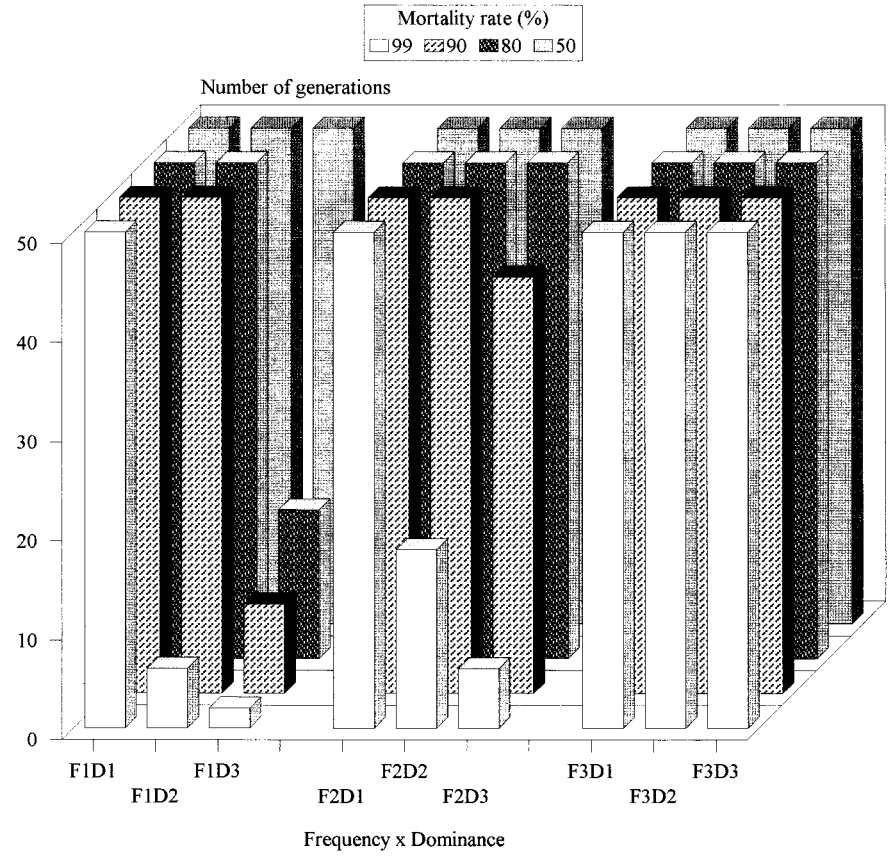

Fig. 13. Number of generations for evolution of insecticide resistance considering level 1 of action level, and level 2 of migration and reproductive disadvantage. Case study 5.

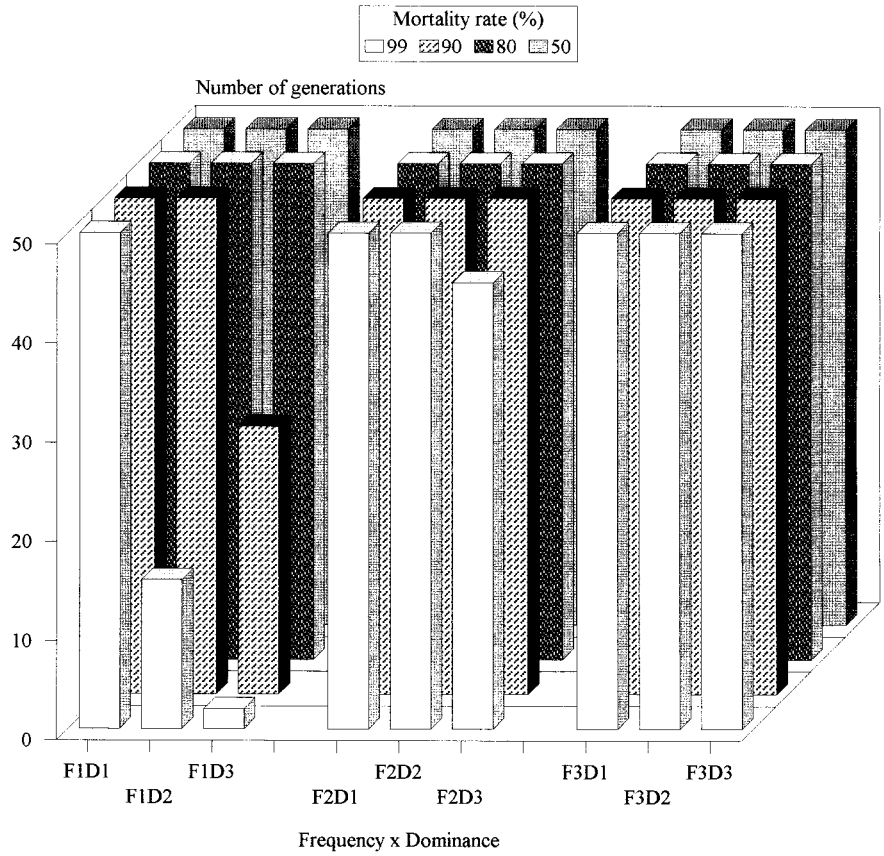

Fig. 14. Number of generations for evolution of insecticide resistance considering level 1 of action level, migration and reproductive disadvantage. Case study 6.

Reducing the action level to the lower level, and maintaining intermediate levels of migration and reproductive disadvantage allows the development of resistance with some combinations containing higher and intermediate genetic frequency (Fig. 13). This effect is still evident when higher migration and reproductive disadvantage are introduced, as in Fig. 14, demonstrating the importance of adequate action levels to help avoiding development of insect resistance.

4 CONCLUSIONS

Results of the simulation model were quite similar to empirical field and laboratory observations, and equivalent to studies made with other insects. Management of possible resistance occurrence should be devised according to specific conditions.[28] Among genetic factors, it is not possible to deal directly with number of genes, dominance and other chromosomal processes (crossover, mutagenesis, genic interaction, gene amplification, multiplicative and additive effects, etc.). The use of insecticides with different mode of action would interfere with the R frequency (or R_1, R_2, R_3, R_n series) reducing and altering the selection pressure. In considering the behavior of the velvetbean caterpillar in the field, we are not concerned with isolated farms, but with macro-regional contexts, so different growers would use different insecticides for controlling the same field generation of the insect. Intermediate to intense migration of moths will rearrange the genetic pool, counterbalancing the selection pressure. Mortality rate was considered the major factor in this study, but the present official Brazilian soy-IPM recommendation instructs farmers to use a field rate providing 80% insect mortality, thus limiting the selection pressure. By running the model, an increase of 18 generations for evolution of resistance was observed in changing from 99 to 80% mortality rate levels. This period of time is normally enough to modify the pesticide market profile, altering the direction of selection pressure. Also, mortality rate can be an extended concept, considering its integration over time, meaning that the use of less-persistent insecticides will avoid unnecessary pressure on low populations of the subsequent generations of the insect.

Although it is not possible to act directly on migration rate, maintenance of field refugia for the insect, like woods or grasses, leaving small plots inside soybean fields unsprayed, or basin management of spraying, avoiding the use of the same variety or date of planting on contiguous farms, will desynchronize insect field peaks and the need for migration control.

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